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藏药椭圆叶花锚的胚胎学*

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摘要 本文首次报道了藏药椭圆叶花锚的胚胎学特征。花药四室，药壁发育为双子叶型；绒毡层异型起源，腺质型绒毡层。小孢子母细胞减数分裂为同时型，四分体的排列方式为四面体型；成熟花粉为3-细胞。子房2心皮，而二心皮连接处强烈膨大、内凸，4列胚珠。薄珠心，单珠被，直生胚珠。胚囊发育为蓼型。胚乳发育为核型。胚胎发育为茄型酸浆I变型。反足细胞在胚囊成熟时期宿存。承珠盘发达。果实成熟时，种子只发育至球型胚阶段。比较了该种与龙胆族其它属、种的胚胎学特征，发现它们大部分特征是相似的，但在如下3个特征上存在区别：子房二心皮连接处强烈膨大、内凸；直生胚珠；具有发达的承珠盘。其胚胎学特征的系统学和分类学意义有待进一步比较与评价。

关键词 藏药，椭圆叶花锚，胚胎学
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Embryology of a Tibetan Medicine *Halenia elliptica*

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Abstract The present paper firstly reports the development of microsporangium, male gametophyte, megasporogenesis, female gametophyte and embryogeny of *Halenia elliptica*, a famous Tibetan medicine. The anther is tetrasporangiate and its wall development conforms to the dicotyledonous type and comprises of epidermis, endothelium, two layers of middle cells and tapetum at the mature stage. The tapetum has dual origin and is similar to the glandular type. Cytokinesis is of the simultaneous type and microspore tetrads are mainly tetrahedral. Pollen grains are mainly 3-celled when shed. The ovary is bicarpellate. The fused margins of two carpels enlarge and intensively protrude into the ovary locule with four lines of ovules. The ovule is unitegmic, tenuinucellate and orthotropous. The development of embryo sac is of the *Polygonum* type. The development of endosperm conforms to the nuclear type and the embryogeny corresponds with the *Physalis* I variation of *Solanad* type. A massive hypostase tissue exists from the 2-nucleate embryo sac to the polycellar proembryo stage. Three antipodal cells persist at the mature embryo stage. The embryo is at the late globular stage when seeds released from the capsule. Most embryological characters are similar when compared with other taxa in Gentianinae. However, it differs from them in three distinctive embryological traits: enlargement and protruding of the fused margins of two carpels into the ovary locule; orthotropous ovules and a

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developed hypostase. However, its systematic and taxonomic significance needs more comparison and revaluation.

Key words *Halenia elliptica*, Embryology, Tibetan medicine

In Qinghai – Xizang Plateau, with a cold climate and short growing season, many ephemeral or annual species must complete their life cycle within a few weeks. It is a mystery why and how these plants acquire their reproductive adaptations to the arid plateau and normally produce their offspring. Up to now, few studies were taken to elucidate the reproductive patterns and recruitment of these alpine plants. The ephemeral annual Gentianaceae species are widely distributed in the plateau mountain as well as its perennial species, which comprises "three famous flowers in the alpine mountains" with primroses and rhododendrons. Gentianaceae species are ideal taxa for studying the reproductive patterns, adaptations and evolution of the Qinghai – Xizang Plateau plants. Furthermore, most species of Gentianaceae are used as important Tibetan medicines. The wild resources decrease annually with the rising collections. It is necessary to propagate the wild species and to conserve the wild resources. However, these practical uses must be based on the well knowledge of natural reproductive patterns of these species.

Halenia elliptica, an effective Tibetan medicine to cure liver diseases, is distributed in the Qinghai – Xizang Plateau and its adjacent area. This annual species relies on the seeds to reproduce in natural conditions. But during the propagation of this species, it is difficult to germinate seeds. The present paper report the embryology of *H. elliptica* for the first time, with a hope to provide a basis for investigating its reproductive pattern and adaptation to the Qinghai – Xizang Plateau. Moreover, based on the embryological information, we intended to discuss the systematic position of *Halenia*.

1 Materials and Methods

Buds, flowers and fruits at different stages of development were collected from Menyuan County of Qinghai Province and fixed in the formalin – acetic – alcohol (FAA). After being staining with Ehrlich' s hematoxylin diluted solution for three days, materials were dehydrated and embedded in Paraffin wax. Serial sections were cut 6 – 10 μm in thickness. Observation and photographs were progressed on Olympus BH2 microscope.

2 Observation and Result

2.1 Microsporangium, Microsporogenesis and Male gametophyte

The anther is tetrasporangiate. The anther wall comprises basically five to six cell – layers: an epidermis, an endothecium, two middle layers, and tapetum (Plate I: 3). The middle layers have a common histogenetic origin with the endothecium (Plate I: 2); the wall formation conforms to the dicotyledonous type. The epidermis persists at maturity and develops fibrous thickenings as well as the

endothecium (Plate I: 10). The middle layers are ephemeral and become crushed during meiosis stage in pollen mother cells. The tapetum is of dual origin, mainly coming from the primary parietal layer, and partly from the ground tissue near the connective tissue (Plate I: 2). Some tapetal cells contain 2 ~ 3 nucleate and show radial elongation from the very early stage of differentiation, and protrude in the anther locule (Plate I: 3 ~ 4). All the tapetal cells degenerate at their original sites and the degenerating tapetum nuclei in the middle of the anther locule (Plate I: 9) are from the early differentiation protruding of thetapetum (PlateI: 3 ~ 4); therefore, the tapetu is similar to the glandular type.

Microsporocytes (Plate I: 3) originating from the primary sporogenus layer and the secondary sporogenous cells (Plate I: 1 ~ 2) undergo meiosis resulting in tetrahedral microspore tetrads (Plate I: 4 ~ 6) by centripetal furrowing. The microspores separating from the tetrad divide to form a large vegetative cell and a smaller generative cell (Plate I: 7). The generative cell undergoes a further division resulting in two sperms (Plate I: 8). Pollen grains are shed at this stage.

2.2 **Megasporangium and female gametophyte**

The ovary is superior and bicarpellary syncarpous. The fused margins of two carpels enlarge and intensively protrude into the ovary locule with four lines of ovules (Plate III: 9). Ovules are tenuinucellar (Plate II: 1), unitegmic and orthotropous (Plate II: 10). A single hypodermal archesporial cell is differentiated and functions directly as the megasporocyte (Plate II: 1). The megasporocyte undergoes meiosis to form a linear tetrad and the chalazal one becomes functional while the rest three degenerating (Plate II: 2). An eight – nulceate female gametophyte of the *Polygonum* type results from three successive free – nuclear divisions and enlargement of the functional megaspore (Plate II: 3 ~ 6). The egg apparatus consists of a pear – shaped egg and two synergids (Plate II: 7). Two polar nuclei fuse at the nearby area of the apparatus (Plate II: 8).

Three antipodal cells of *H. elliptica* were not ephemeral as observed in most angiosperms (Hu, 1982). They persist at the mature embryo sac stage (Plate II: 7 ~ 8). Afterward, three cells enlarge considerably and each of them has a prominent nucleus and dense cytoplasm (Plate III: 9). They begin to degenerate at the division of the zygote and are completely absorbed at the polycellar proembryo stage.

A hypostase exists from the two – nucleate embryo sac stage to polycellar proembryo stage. It contains a group of special cells with dense cytoplasm and very thickened walls (Plate III: 9). The hypostase borders directly on the persistent antipodal cells after the gametophyte is mature. It begins to degenerate after all antipodal cells are absorbed. We guess the hypostase is related to absorbing and transporting of the nutrition to the embryo sac from the integument.

2.3 **Endosperm and Embryo**

The development of the endosperm is of the nuclear type (Plate III: 8). The zygote divides transversely to form a terminal cell and a basal cell (Plate III: 1 ~ 3). Both the terminal and the basal cell undergoes a further division resulting in a linear proembryonal tetrad (Plate III: 4). The eight – celled proembryo consists of a linear of eight cells from the third transverse division of the tetrad (Plate III: 5). Four cells from the basal cell undergo a vertical divisions, to form a two seriate suspensor

(Plate III: 6 ~ 7). The four cells from the terminal cells contribute to the development of the entire embryo. Therefore, the embryogeny conforms to the *Solanad* type (Hu, 1982). The third generation of the zygote consists of linear eight cells, and the cells from the top cell of the tetrad at last contribute to the formation of the stem tip and cotyledons; therefore the variation type is similar to the *Physalis* variation of *Solanad* type (Johanson, 1950).

Most seeds are shed from the capsule at the globular to heart – shaped embryo stage (Plate III: 7). Before germinating, seeds must undergo a post maturing. The seed contains abundant endosperm cells. Only the epidermis of the integument becomes the seed coat and the inner layers are all crushed and absorbed. The time's span between flowering to shedding seeds amounts to no more than 20 days.

3 Discussion

(1) The present study reveals the annual *H. elliptica* with persistent antipodal cells and developed hypostase, which may account for its speedy seeds development under the arid surrounding of the Qinghai – Xizang Plateau. The time' s span in *H. elliptica* between flowering to shedding seeds amounts to no more than 20 days. It is easy for it to produce normal seeds and complete recruitment of offspring in spite of short growing season in the arid habits of the plateau. Akhalkatsi and Wagner (1997) found similar results in the arid Alps. mountain, that the persistent antipodal cells of the annual gentians accelerate the embryogenesis and shorten the time of seeds developing. Because of the short growing season, the ephemeral plants in the alpine mountains must acquire some peculiar traits to help them to complete their life histories within such short time. As for Gentianaceae plants, most annual species have persistent antipodal cells to help them accelerate seeds developing (Liu and Ho, 1997; 1996a, b). It might be one of reasons why so many ephemeral Gentianaceae species distribute widely on the arid mountains especially on the arid Qinghai – Xizang Plateau. Furthermore, we found the difficulty of germinating and propagating of this Tibetan medicine exists in its post maturing of seeds.

(2) Most embryological features of *H. elliptica* are similar to those found in other taxon and conform to the embryological framework of Gentianinae (Liu and Ho, 1997; 1996a, b; Rao and Nagara, 1982): tetrasporangiate anthers, dicotyledonous type of the anther wall, glandular tapetum, simultaneous cytokinesis in the microsporocytes, two – or three – celled pollen, unitegmic, tenuinucellar ovules, *Polygonum* type of the embryo sac, nuclear endosperm, *Solanad* type of embryogeny and endosperm cells seeds.

However, *H. ellipitica* displays three distinctive traits of its own, which are unique in Gentianinae and of systematic significance. Firstly, the fused margins of two carpels enarlge and intensively protrude into the ovary locule. Secondly, all ovules are orthotropous different from the anatropous ovules reported in the other taxa. Thirdly, a hypostase tissue exists from the two – nucleate embryo sac to polycellar proembryo stage.

All these embryological characters are thought as evolutionary, for they are uncommon in the an-

giosperms and might derive from more common states with more special functions (Tobe, 1989). The results of this embryological study indicate *Halenia* might be at a more evolutionary stage than other genera in Gentianinae. Ho and Liu (1990) suggested *Halenia* have a close relationship to *Swertia* and more evolutionary than it based on the analyses of the gross morphology. Except for common embryological features for all Gentianinae taxa, no obvious embryological connection is found between *Halenia* and *Swertia* when compared with embryology of several *Swertia* species reported by (Rao and Nagara, 1982; Rao, 1977) and Maheswari and Laksjminarayana (1975). ITS sequence phylogeny showed *H. elliptica*, *Swertia tetraptera* and *S. speciosa* formed a monophyletic clade while *S. frachetiana*, a more typical species in *Swertia*, nested in *Gentianella* - *Comastoma* branch (Yuan and Kupfer, 1995). *S. tetraptera* and *S. speciosa* were thought to represent two distinct genera (*Anagalidium* and *Frasera*) delimited from *Swertia* by different authors (Pringle, 1978; Ho and Shih, 1976). Molecular data implied that, in order to make the genus *Swertia* monophylogenetic, two genera should be separated and excluded from *Swertia* unless *Halenia* would be included in *Swertia*. Nevertheless, up to now, this inferring haven't been supported by other evidences. If the unique embryological characters of *H. ellipitica* are also found in *S. tetraptera* and *S. specioa*, the taxonomic treatment of *Swertia* and the systematic relationships of genera in Gentianinae may really need to be reevaluated.

References

Akhalkats M, Wagner J, 1997. Comparative embryology of three Gentianaceae species from the central Caucasus and the European Alps. *Pl Syst Evol*, **204**: 29 ~ 48

Davis G L, 1966. Systematic Embryology of the Angiosperms. New York: John Wiley & Sons Inc.

Ho T N, Liu S W, 1990. The infrageneric classification of *Gentiana* (Gentianaceae). *Bull Br Mus Nat Hist* (*Bot*), **20**: 169 ~ 192

Ho T N, Shih W L, 1976. Qing Xie Dan and Dau Da - - Chinese medical herbs for treating hepatitis. *Acta Phytotax Sin*, **14** (2): 65 ~ 67

Hu S Y, 1982. Embryology of Angiosperms. Berjing, People Education Press, 80 ~ 102

Johansen D A, 1950. Plant Embryology. Massushusetts: Waltham Press

Johri B M, Ambegaokar K B, Srivastava P S, 1992. Comparative Embryology of Angiosperms. Berlin, Herdelberg, New York, Springer.

Liu J Q, Ho T N, 1997. Embryology of *Gentianopsis paludosa* Ma. *Acta Biol Plateau*, **13**: 31 ~ 41

Liu J Q, Ho T N, 1996a. The embryological studies of *Comastoma pulmonarium* (Gentianaceae). *Acta Phytotax Sin*, **34** (6): 577 ~ 585

Liu J Q, Ho T N, 1996b. Embryological studies of *Gentianella azurea* (Bunge) Holub. *Acta Bot Yunnanica*, **18** (2): 151 ~ 157

Maheswari D H, Laksjminarayana, 1977. Embryological studies in Gentianaceae. *J Indian Bot Soc*, **56**: 182 ~ 188

Pringle J S, 1978. Sectional and subgeneric names in *Gentiana* (Gentianaceae). *Sida*, **7**: 232 ~ 247

Rao S K, 1975. Gametophytes and endosperm development in *Swertia corymbosa*. *Wt Acta Bit Indica*, **3**: 165 ~ 166

Rao S K, Nagara J M, 1982. Studies in Gentianaceae: embryology of *Swertia minor* (Gentianaceae). *Can J Bot*, **60**: 141 ~ 151

Tobe H, 1990. The embryology of angiosperms: its broad application to systematic and evolutionary study. *Bot Mag* (Tokyo), **102**: 351 ~ 367

Yuan Y M, Kupfer P, 1995. Molecular phylogenetics of the subtribe Gentianinae (Gentianaceae) inferred from the the sequences of internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Pl Syst Evol*, **196**: 207 ~ 226

Explanation of Plates

ANT. Antipodal cells; DM. Degenerated megaspores; E. Egg cell; EP. Epidermis; EN. Endothecium; M. Middle layer; PN. Polar nucleus; Sp. Sperm. Sy. Synergid cell; T. Tapetum;

Plate I 1. Archesporial cells (arrow). 2. The primary parietal layer dividing to form an outer endothelium and a middle layer, the arrow showing the tapetum originating from the ground tissue near the connective tissue. 3 ~ 4 Anther wall, and the arrow indicating the elongating and protruding of the 2 ~ 3 nucleate tapetum cells. 5. Anaphase II of meiosis in microsporocytes. 6. A tetrahedral microspore tetrad. 7. A bicellular pollen grain showing the vegetative and generative cells. 8. A 3 – celled pollen grain. 9. Showing the degenerating of the tapetum cells at the single – nucleate stage, noting the central tapetum cells (arrow) being from the early differentiatipn protruding (see 3 ~ 4). 10. The persistent epidermis as well as thickened endothelium. (1 ~ 3 × 652; 4 × 560; 5 ~ 8 × 1630; 9 ~ 10 × 400).

Plate II 1. A megaspore mother cell. 2. A single nucleate embryo sac, noting three degenerated megaspores. 3. A 2 – nucleate embryo sac. 4. A 4 – nucleate embryo sac. 5 ~ 6. Successive sections of a 8 – nucleate embryo sac. 7 ~ 8. Successive sections of a mature embryo sac. 10. Showing the intensive protruding of the fused carpels and four lines of ovule. (1 ~ 6 × 652; 7 ~ 8 × 344; 9 × 100).

Plate III 1. A zygote. 2. The dividing of the zygote 3. A 2 – celled proembryo; 4. A linear 4 – celled proembryo. 5. A linear 8 – celled proembryo. 6. The vertical dividing of the 8 – celled proembryo cells. 7. A early globular embryo when seeds shed. 8. Showing nuclear endosperm. 9. Showing the hypostase and persistent antipodal cells at the mature embryo sac. 10. Showing the unitegmic and orthotropous ovule. (1 ~ 5 × 652; 6 ~ 8 × 200; 9 × 400; 10 × 136).

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Kalkman C, 1988. The phylogeny of the Rosaceae. *Bot Jour Linn Soc*, **98**: 37 ~ 59

Oliver, 1886. Plagiospermum sinense. *Hooker's Icog Plant*, **16**: t.1526

Rehder, 1915. Subfam. Prunoideae. Sarg. Pl. Wils. 2: 344 ~ 345

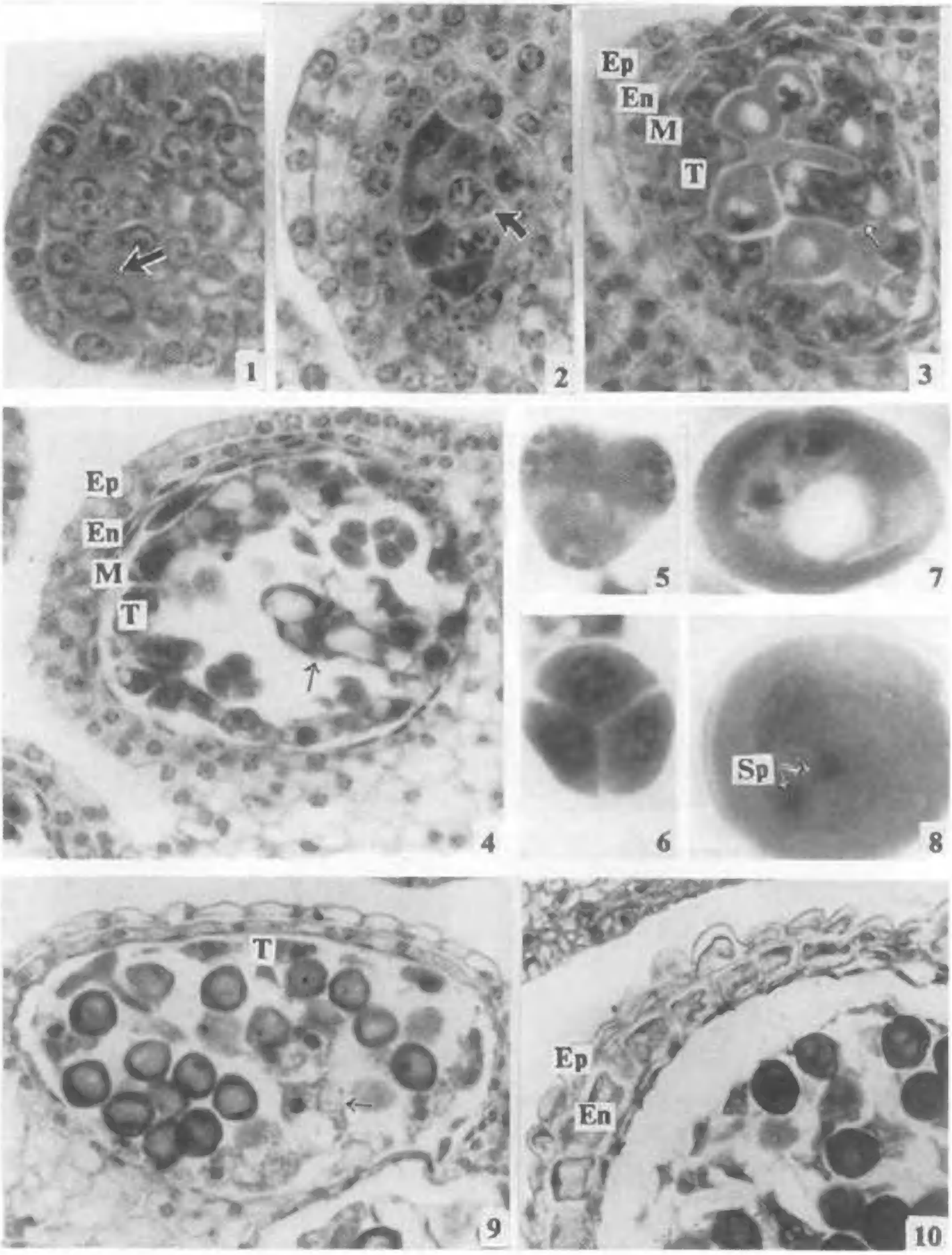
Rauschert, 1982. Nomina nova generica et combinationes novae spermatophytorum et pteridophytorum. *Taxon*, **31** (3): 561

Stebbins G L, 1958. On the hybrid origin of the Angiosperms. *Evolution*, **12**: 267 ~ 270

Stering, C, 1963. The affinities of Prinsepia (Rosaceae) . *Amer J Bot*, **50**: 693 ~ 699

Takhtajan A L, 1995. Dictionary of generic names of seed plants. Columbia Univ. PressTakhtajan, A L, 1997. Diversity and classification of flowering plants. Columbia Univ. Press

Zhang S Y, 1992. Systematic wood anatomy of the Rosaceae. *Blumea*, **37**: 81 ~ 158



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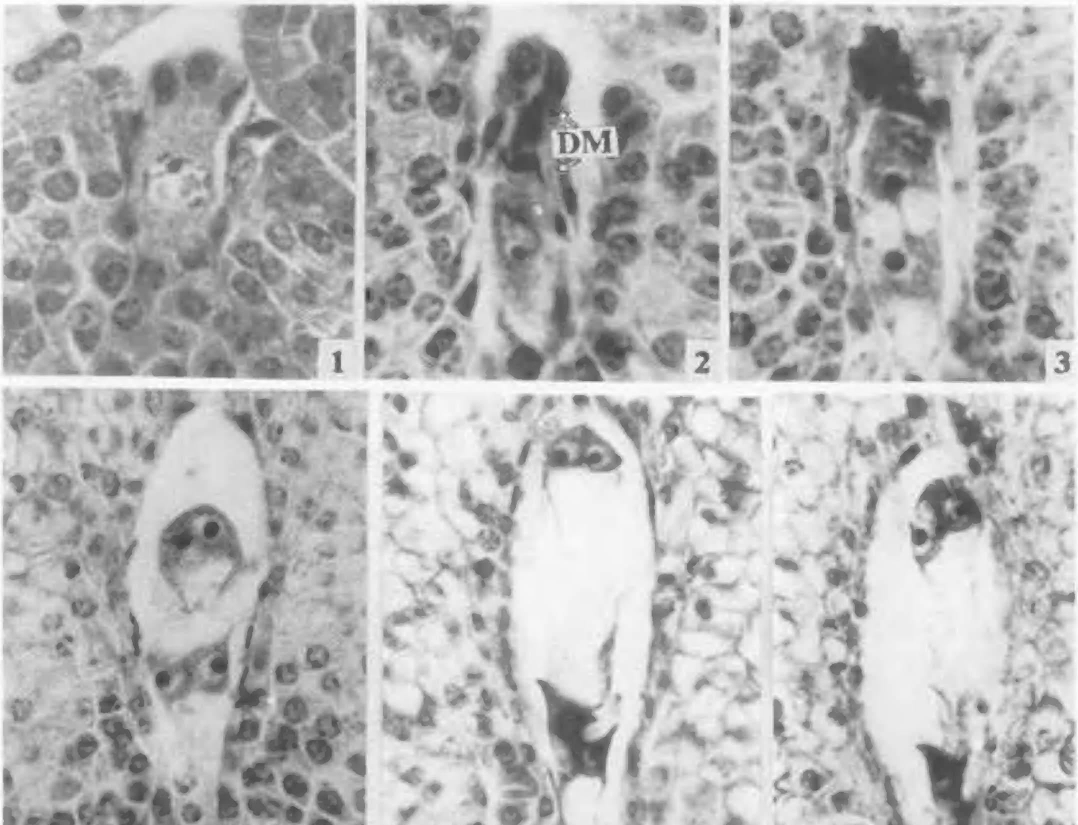

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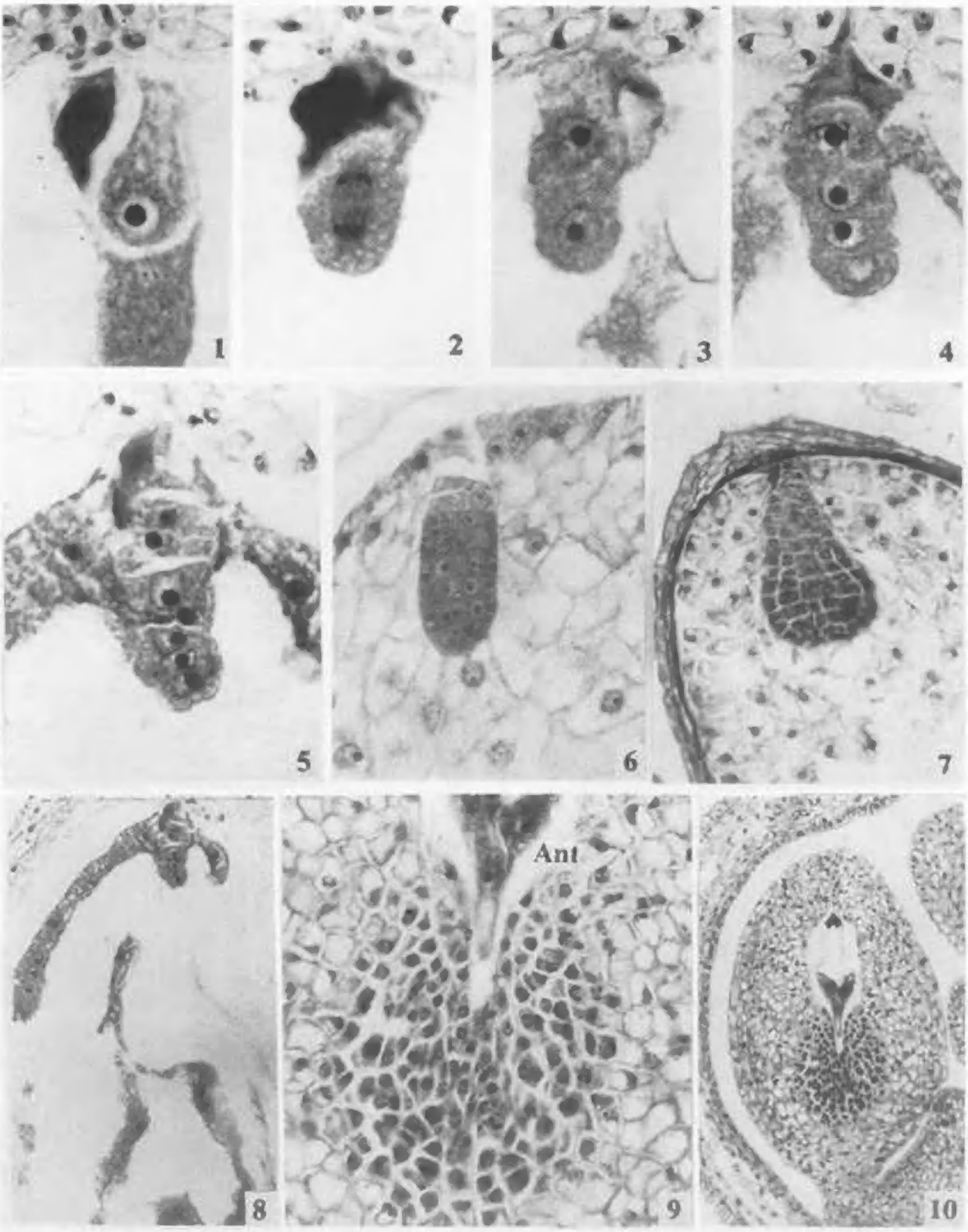

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